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Phytochemical Composition and Antioxidant Activity of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* Used against Seed-borne Fungi in Burkina Faso

Léon W. Nitiema[1*], Pierre A. E. D. Sombié[1], Moumouni Koala[2] and Antonella Del Fiore[3]

[1]Centre National de la Recherche Scientifique et Technologique (CNRST), Institut de l'Environnement et de Recherches Agricoles (INERA), 01 BP 476, Ouagadougou 01, Burkina Faso.
[2]Centre National de la Recherche Scientifique et Technologique (CNRST), Institut de Recherche en Sciences de la Sante´(IRSS), 03 BP 7047, Ouagadougou 03, Burkina Faso.
[3]Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Via Anguillarese 301 - 00123 Roma, Italy.

Authors' contributions

This work was carried out in collaboration among all authors. Author LWN designed the study, managed the literature searches. Authors ADF and LWN made the first phytochemical screening. Authors PAEDS and MK conducted the bench work. Author LWN wrote the first draft of the manuscript. Author PAEDS performed the statistical analysis. Authors LWN, PAEDS, MK and ADF corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* from Burkina Faso were investigated for their phytochemical composition and their antioxidant activities.
Methods: High-performance thin-layer chromatography (HPTLC) method was used for phytochemical screening. The total phenolic, total flavonoid and anthocyanin contents of extracts

**Corresponding author: E-mail: leon.nitiema@gmail.com;*

were assessed. The antioxidant potentials of the extracts were also evaluated using 2,2-diphenyl-l-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP).
Results: Phenolic compounds, flavonoids and anthocyanins were present in all these plant extracts. Tannins were only found in *Acacia gourmaensis* extract. *Acacia gourmaensis* extract exhibited the highest total phenolics (807.58 ± 28.63 mg GAE/g), total flavonoids (271.39 ± 58.46 mg QE/100 g), total anthocyanins (83.16 ± 0.14 µg/g) contents and had the highest antioxidant activity by DPPH (330.84 ± 16.23 µg AAE/g) and FRAP methods (3211.11 ± 52.24 µg AAE/g). *Balanites aegyptiaca* and *Securidaca longepedunculata* showed the lowest phenolic compounds (80.72 ± 2.11 mg GAE/g and 76.69±1.84 mg GAE/g respectively); total flavonoids (88.7 ± 1.65 mg QE/100 g and 104.54 ± 9.65 mg QE/100 g respectively), anthocyanins (24.49±1.43 µg/g and 24.57±0.52 µg/g respectively) contents and had the lowest antioxidant activity for DPPH method (46.83 ± 3.01 and 56.20 ± 3.79 µg AAE/g respectively) and FRAP method (102.06 ± 5.09 and 57.78 ± 0.99 µg AAE/g respectively).
Conclusion: *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* represent natural sources of phenolic antioxidant *compounds* that can be used as a bio-*fungicide.*

Keywords: Balanites aegyptiaca; Securidaca longepedunculata; Acacia gourmaensis; phytochemical compounds; antioxidant activity; antifungal activity.

1. INTRODUCTION

Fungi are the cause of important crop diseases. Many of these pathogens are carried on or inside seeds and can reduce seed germination and seedling emergence [1]. Seed-borne pathogens may cause seed abortion, seed rot, seed necrosis, reduced or eliminated germination capacity as well as seedling damage [2].

Plant extracts are considerable natural sources of antimicrobial compounds for the control of human and plant diseases [3]. Natural plant products are an important source of new chemicals in agriculture [4]. Plant-derived pesticides are available and cost effective in countries where synthetic pesticides are expensive [5]. A considerable number of natural products and medicinal plants contain some active phytochemical ingredients such as phenolic compounds, flavonoids, alkaloids, tannins, coumarins, curcuminoids or terpenes that induce various biological activities in animals including antioxidant, anti- inflammatory and anti-cholinesterase effects [6,7]. Plants used for their antifungal properties in Burkina Faso include *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* [8-10].

Balanites aegyptiaca (L.) Delile, known as desert dates belonging to the Zygophyllaceae family, is one of the most common wild plant species in the drylands of Africa and South Asia [11]. The plant (leaves, roots and bark, fruits) is used in phytotherapy for its potential antimicrobial effect [12]. Almost all parts of this plant are used in traditional medicine for anti-inflammatory, anti-helminthic, insecticidal, anti-molluscicidal, anti-fungal, anti-bacterial activities [12]. It is traditionally employed in the treatment of jaundice, yellow fever, syphilis, diarrhea, epilepsy, cough and wound healing [13].

Securidaca longepedunculata Fres, commonly known as violet tree, is a savanna grown medicinal plant belonging to the Polygalaceae family. It is commonly used as a medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache and various other inflammatory conditions [14]. Dried roots powder is also used as a pest control agent in storage, and methanol extracts of the roots have the potential to protect against insect pests and microbial agents [15,16].

Acacia gourmaensis A. Chev. is the member of the Fabaceae family known in Burkina Faso. The aqueous extract of this plant is used for antifungal activities [17].

In recent studies, *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* hydro-ethanolic extracts are used against seed-borne fungi in Burkina Faso [9,10]. It is, therefore, necessary to study the phytochemistry and biological properties of these plant extracts to confirm their antifungal activity.

The aim of the present work was to evaluate the phytochemical composition as well as the antioxidant activity of hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis*.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh stem bark of *Balanites aegyptiaca* (Ba), *Securidaca longepedunculata* (Sl) and *Acacia gourmaensis* (Ag) was harvested from different trees during May 2018 in Mogtedo localized in the Plateau-Central region of Burkina Faso. The plant material was washed with tap water to remove debris and dust particles and then rinsed with sterile distilled water. It was after dried under shade at 25°C, pulverized with a pestle and mortar, finally kept in a sterile transparent polyethylene bag and stored at 4°C until used.

2.2 Preparation of Extract

Fifty grams (50 g) of the plant material powder were extracted by using 500 mL of ethanol (70%) under mechanical agitation at room temperature during 24 hours. The mixture was filtered, concentrated and lyophilized by using a freeze-drying system to give the hydro-ethanolic extract.

2.3 Phytochemical Composition

2.3.1 High-Performance Thin-Layer Chromatography (HPTLC) screening

Phytochemical screening of standard solution and samples (Sl, Ba and Ag) extracts was performed on $20\,\mathrm{cm} \times 10\,\mathrm{cm}$ silica gel $60\,\mathrm{F}_{254}$ HPTLC (glass) plate (Merck, Darmstadt, Germany). 2 µL of each extract were applied as 5 mm bands with a semi-automatic plate spotter (CAMAG, Linomat V, Switzerland) set to dispense along a line 10 mm from the bottom edge of the plate. The distance between tracks was 10 mm. Distances from left and right edge of the plate were 20 mm. The plates were placed in a $20 \times 20\,\mathrm{cm}$ vee-bottomed TLC tank (saturation time 30 min) containing ethyl acetate: formic acid: acetic acid: water (100:11:11:26) and ethyl acetate: water: methanol: n-hexane (11.9:1.6:1.4:3.5) respectively for flavonoids and tannins. The developed plates were then dried with an air dryer (cold air) for 5 min. Concerning flavonoids, the plate was heated at 105ºC for two (02) min and sprayed with the Neu reagent. Evaluation was performed under UV 366 nm. As for the tannins, the plate was sprayed with a 2% $FeCl_3$ reagent. Evaluation was performed under white light.

2.3.2 Determination of total phenolic content

Total phenolic content was determined, according to Singleton, et al. [18]. Different plants extracts (25 µL, 100 µg/mL in Methanol) were mixed with Folin Ciocalteu reagent (105 µL, 0.2 N) and 5 min later with sodium bicarbonate (100 µL, 75 g/L). After 1 hour incubation, the absorbance of each mixture was measured at 760 nm against a blank with a microplate reader. A standard calibration curve was plotted using Gallic acid (0-100 mg/L). Polyphenol content was expressed as mg of Gallic acid equivalent per g of extract (mg GAE/g).

2.3.3 Determination of total flavonoids content

The total flavonoids content was estimated according to the method of Dowd as adapted by Arvouet–Grant, et al. [19]. Different extracts of plants (75 µL, 100 µg/ mL) were mixed with aluminum trichloride (75 µL, 2% in methanol). Absorbances were read at 415 nm after 10 min of incubation against a blank using a microplate reader. Total Flavonoids content was expressed as mg of quercetin equivalent per g of extract (mg QE/g) using a standard calibration curve of quercetin (0-150 mg/L).

2.3.4 Total anthocyanins assays

Anthocyanin contents of samples were carried out by spectrophotometer (SHIMAZU) following the pH-differential method [20]. Two buffers were used in this method: potassium chloride pH1.0 (0.025 M) and acetate buffer pH4.5 (0.4 M). Briefly, 0.2 mL of sample extract wasadded to 1.8 mL of each buffer. Each mixture was read against a blank at 510 and 700 nm. Absorbance (A) was evaluated following the formula:

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$$

The concentration of total monomeric anthocyanins in the sample was determined according to the calculation of cyanidin-3-glucoside concentration below:

$$TAC\ (mg/L) = \frac{(A \times M \times FD \times 1000)}{(\varepsilon \times l)}$$

A: Absorbance; M: Molecular Weight; (449.2); DF: Dilution Factor; ε: Molar Absorptivity (26900).

2.4 Antioxidant Activity

Antioxidants are capable of deactivating free radicals through two main mechanisms: individual electron transfer (ET) and hydrogen atom transfer (HAT). In this work antioxidant activities of plant extracts were evaluated by DPPH assay, classified as a HAT-method and FRAP assay, classified as ET-method.

2.4.1 DPPH radical scavenging activity

The ability of plant extracts to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was evaluated at 517 nm, as described by Sombié, et al. [21]. The DPPH Radical Scavenging Activity was expressed as µg of ascorbic acid equivalent per g of extract (µg AAE/g of extract).

2.4.2 Ferric Reducing Antioxidant Power (FRAP) assay

The ability of the plant extracts to reduce iron (III) to iron (II) was measured at 700 nm following the procedure described by Sombié, et al. [21]. Iron (III) reducing activity was determined as µg quercetin equivalent per g of dry seed extract (µg QE/g of extract).

3. RESULTS

Three local plants, *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* from Burkina Faso used as antifungal extracts were studies to confirm their biological activities. The results obtained are described below.

3.1 Phytochemical Composition

3.1.1 Phytochemical screening by HPTLC

Phytochemical screening of hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* results were shown on the chromatoplates (Fig. 1). The HPLC chromatographic profile showed blue, green and fluorescence spots under ultraviolet 366 nm revealing the presence of flavonoids in the hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* (Fig. 1a). Indeed, after heating (105°C for 5 min) and after spraying of Neu reagent, flavonoids appear blue, green, yellow, orange on the plate. In additional, after spraying with the 2% $FeCl_3$ reagent, the chromatoplate presented a much more intense brown spot in the hydro-ethanolic extract of *Acacia gourmaensis* compared to the hydro-ethanolic extracts of *Balanites aegyptiaca* and *Securidaca longepedunculata* in the visible (Fig. 1b). The brown coloration is characteristic of the tannins.

3.1.2 Phytochemical contents

Phenolic compounds, flavonoids and anthocyanins contents of hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* were performed by spectrophotometric method. Results of phenolic compounds, flavonoids and anthocyanins contents were showed by Fig 2. The total polyphenol content of *Acacia gourmaensis* hydro-ethanolic extract was 807.58 ±28.63 mg GAE/g and showed a highly significant difference ($P < 0.05$). *Balanites aegyptiaca* and *Securidaca longepedunculata* had low total phenolic contents of 80.72±2.11 and 76.69±1.84 mg GAE/g extract, respectively. Flavonoid contents were ranged from 271.39±58.46, 104.54±9.65 to 88.70±1.65 mg QE/100 g of extract respectively for *Acacia gourmaensis, Securidaca longepedunculata, and Balanites aegyptiaca*. *Acacia gourmaensis* hydro-ethanolic extract contained significantly higher total flavonoid contents ($P < 0.05$) than the two other plants. Anthocyanin contents in our hydro-ethanolic plant extracts were 83.16±0.14, 24.57±0.52 and 24.49±1.43 µg/g respectively for *A. gourmaensis, S. longepedunculata and B. aegyptiaca*. *Acacia gourmaensis* extract contained a significantly higher content of anthocyanin among all the other plants ($P < 0.05$).

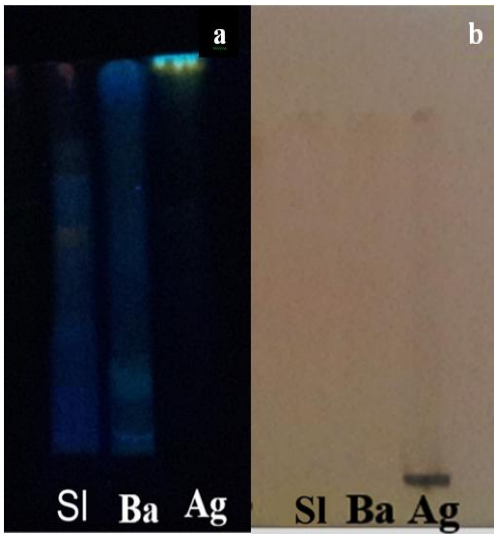

Fig. 1. Phytochemical screening of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* (a): flavonoids, (b): tannins

Sl: Securidaca longepedunculata; Ba: Balanites aegyptiaca; Ag: Acacia gourmaensis

3.2 Antioxidant Activity

The antioxidant activities of three plant extracts analyzed using the free radical scavenging capacity 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP)

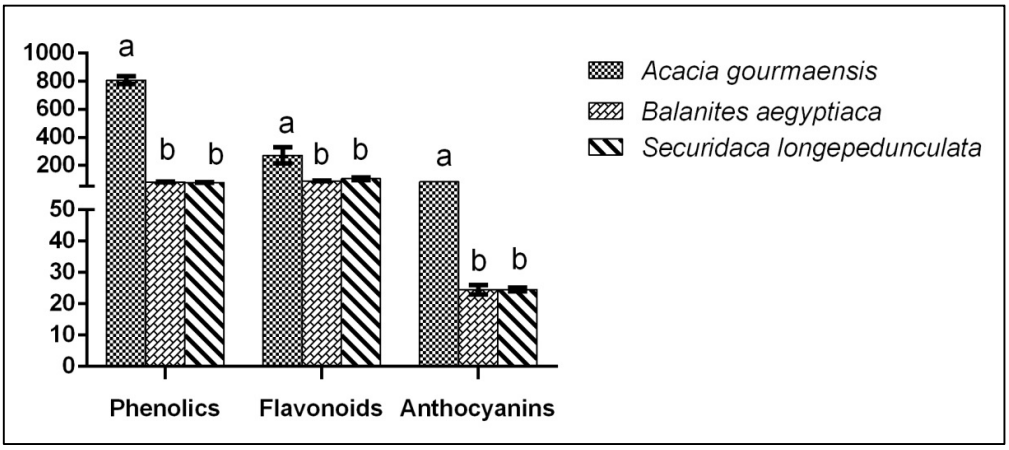

Fig. 2. Phytochemical content of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis*

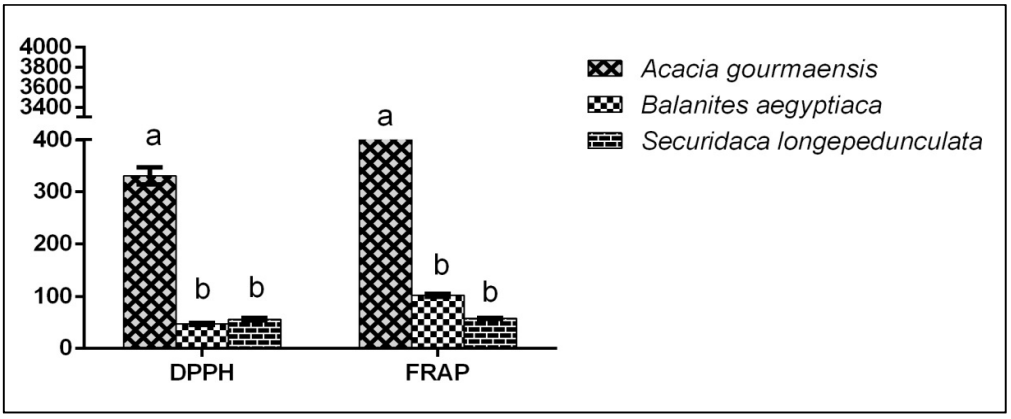

Fig. 3. Antioxidant activity of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis*

methods are showed by Fig. 3. *Acacia gourmaensis* hydro-ethanolic extract showed high DPPH radical scavenging activity (330.84±16.23 µg AAE/g of powder) with a significant difference than two other plants ($P < 0.05$). *Securidaca longepedunculata* and *Balanites aegyptiaca* extract showed low DPPH scavenging activity with 56.20± 3.79 and 46.83± 3.01 µg AAE/g respectively. By the FRAP assay, *Acacia gourmaensis* hydro-ethanolic extract had a significantly ($P < 0.05$) higher reducing power capacity (3211.11±52.24 µg AAE/g of powder) followed by *Balanites aegyptiaca* and *Securidaca longepedunculata* (102.06±5.09 and 57.78±0.99 µg AAE/g of powder, respectively.

4. DISCUSSION

Phytochemical screening revealed the presence of phenolic compounds, particularly flavonoids and tannins, in the hydro-ethanolic extracts of *Balanites aegyptiaca*, *Securidaca longepedunculata* and *Acacia gourmaensis* with different contents. In addition, it is important to note that the spots coloration and the intensity differ from one plant to another on the one hand, and within the same plant on the other plant. Indeed, the blue, green spots observed on the chromatograms profile under UV/366 nm may correspond to several classes of secondary metabolites. Flavonoids are secondary metabolites and are considered to be one of the most common groups of natural constituents found in plants [22]. In this study, flavonoids were found in all these three species with a relative abundance and less differentiation in *Balanites aegyptiaca* and *Securidaca longepedunculata* (Fig. 1). The group of tannins is composed of two classes, the‘‘hydrolysable’’ and the ‘‘condensed’’ tannins according to their chemical structure [23].

The present study reported that tannins are more present in *Acacia gourmaensis* extract (Fig. 1). Several studies have established a relationship between biologic proprieties and phenolic compounds. Kahkonen, et al. [24] stated that flavonoids are probably the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant, antimicrobial activity and free radical scavenging properties. It has been demonstrated that condensed tannins have more antimicrobial properties [25]. Previous studies revealed the presence of tannin only in *Securidaca longepedunculata* leaves extracts [16,26]. However, tannin is seldom found in *Balanites aegyptiaca* extract [13,27]. The effective presence of flavonoids and tannins in the hydro-ethanolic extracts would partly explain the therapeutic proprieties of *Balanites aegyptiaca*, *Securidaca longepedunculata* an *Acacia gourmaensis*.

The dosage of phenolic compounds showed a significative difference ($P < 0.05$) between the hydro-ethanolic extracts of *B. aegyptiaca*, *S. longepedunculata* and *Acacia gourmaensis*. Another investigation in Burkina Faso reported low total polyphenol content (532 mg GAE/g) in stem bark methanolic extract of *B. aegyptiaca* [28]. Muanda, et al. [29] showed that the aqueous methanol root extract of *S. longepedunculata* total polyphenol content was 9.86 mg GAE/g. Previously in Burkina Faso, Karama, et al. [30] were shown that *S. longepedunculata* methanolic extract content more flavonoids in leaves from warm period (40.96±0.19 mg QE/g) than leaves collected in cold period (28.74±0.39 mg QE/g). In other hands, Ouedraogo, et al. [28] founded 14±0.2 mg GAE/g flavonoids in the stem bark methanolic extract of *B. aegyptiaca*. Anthocyanins are attractive compounds due to their biological properties, mainly as antioxidants or antifungal properties [31,32]. Like total phenolics and total flavonoids, the total anthocyanins content of hydro-ethanolic extract of *Acacia gourmaensis* is highest compared to the hydro-ethanolic extracts of *Balanites aegyptiaca* and *Securidaca longepedunculata*.

In this study, two methods (DPPH and FRAP) were used to assess the antioxidant activity of hydro-ethanolic extracts of *B. aegyptiaca*, *S. longepedunculata* and *A. gourmaensis*. In both methods, the hydro-ethanolic extract of *Acacia gourmaensis* showed the highest antioxidant activity of tree plants. This study confirmed the antioxidant activity of *Balanites aegyptiaca* and *Securidaca longepedunculata* as reported by previous workers [29,33] even though their activities are low compared to *A. gourmaensis* hydro-ethanolic extract.

In this study, *Acacia gourmaensis* that have the high total phenolics, total flavonoids and anthocyanins contents also showed the strong antioxidant activity by the DPPH and FRAP methods. The high antioxidant potential of *Acacia gourmaensis* can be explained by the fact that phenolic compounds are capable of donating an electron or hydrogen atom for the reduction of free radicals. The antioxidant activity of the plant extracts can be related to their amount in phenolic compounds or to the structure of phenolic compounds [34,35]. Indeed, they are compounds of one (or more) aromatic rings bearing one or more hydroxyl groups. They are potentially capable of neutralizing free radicals by forming phenoxyls radicals stabilized by resonance [35]. The effectiveness of these compounds is explained by the delocalization of the single electron of the oxygen with the aromatic ring and thus stabilizes the radical formed.

In this study, for the first time, phytochemical composition and antioxidant activity of *Acacia gourmaensis* extract have been examined. Antifungal activity of *A. gourmaensis*, as demonstrated in previous works [10,17], could be explained by presence of phytochemical compounds such as flavonoids, tannins or anthocyanins.

The present study showed that the extracts possessing the highest phenolic contents were also found to have the highest flavonoids, and anthocyanins contents. The same trains were observed with antioxidant activity from the DPPH and FRAP methods. The hydro-ethanolic extract of *Acacia gourmaensis* was the most efficient in phytochemical content and antioxidant activity followed by *Securidaca longepedunculata* and *Balanites aegyptiaca*.

In the view of antimicrobial activity, Junaidu, et al. [26] attributed antifungal effects of *S. longepedunculata* by the presence of the active phytochemicals like flavonoids tannins, saponins, alkaloids and glycosides in the extracts. Recent studies revealed that *S. longepedunculata* hydro-ethanolic extract had an important antifungal activity [10,26]. Likewise, Tula, et al. [27] reported that leaves of *Balanite aegyptiaca* aqueous extracts possessed polyphenols, saponins, steroids, and flavonoids. However,

flavonoids and tannins were not found with *B. aegyptiaca* alcoholic extracts [12,33]. Very recent studies showed antifungal activity of *B. aegyptiaca* [8,10].

Regarding phytochemical content and antioxidant activity, *Acacia gourmaensis* is supposed to have more antifungal activity, as described by other authors in previous studies [6]. However, the opposite was found in our previous study. We have done a comparison of antifungal activity of *Acacia gourmaensis, Securidaca longepedunculata* and *Balanites aegyptiaca.* This study showed that *Acacia gourmaensis* have the lowest antifungal activity followed by *Securidaca longepedunculata* and *Balanites aegyptiaca* [9,10]. Indeed, Demirci, et al. [36] reported that plant extracts antimicrobial properties could be inhibited by certain compounds used as a source of energy by microorganisms. Moreover, antimicrobial activity is more linked to specific molecules and synergy effects of bioactive constituents in plant extracts [37].

5. CONCLUSION

This study has shown that *Acacia gourmaensis*, *Securidaca longepedunculata*, and *Balanites aegyptiaca* from Burkina Faso, have some antioxidant phytochemicals with known antifungal activities. The hydro-ethanolic extracts of *Acacia gourmaensis*, which had the highest total phenolics, flavonoids and anthocyanins contents, were found to possess the strongest radical scavengers in both DPPH and FRAP assays. Nevertheless, the link between phytochemicals contents and antifungal activity of these extracts remains to be proved. The present study provides some information on the phytochemical and antioxidant activity of *Acacia gourmaensis*, *Securidaca longepedunculata* and *Balanites aegyptiaca* which paves the way for further research to identify the active compounds responsible for the biological activity of the plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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